Effect of Increased Supplementation of Vitamin E During Heat Stress

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Introduction

Vitamin E is an antioxidant that plays important roles in the maintenance of cellular membranes, immunity, and reproduction (National Research Council [**NRC**], 2001). The form that is most common in feeds and is most biologically active is α-tocopherol. Unlike vitamin A, it is not thought to be degraded by ruminal microorganisms. A specific requirement for vitamin E has not been defined yet because titration studies are lacking. The recommended rate of supplemental vitamin E is 1.6 and 0.8 IU/kg of body weight for pregnant dry cows and lactating cows, respectively. A 650 kg cow supplemented at the recommended guideline of the 2001 Dairy NRC would consume daily ~1,000 IU prepartum and ~ 500 IU postpartum. Cows fed fresh forages will require less supplemental vitamin E than this. Unlike plasma retinol concentrations, plasma α-tocopherol concentrations do reflect vitamin E intake. Based upon optimizing neutrophil function and minimizing clinical mastitis, the minimal acceptable concentration of plasma α-tocopherol for the dairy cow within a day or two after calving was proposed to be 3 to 3.5 ug/ml of plasma (Weiss, 1998). Cows at later stages of lactation may have a different minimal acceptable concentration as they may be under less immunological stress. Life changes that increase metabolic demands, such as parturition and copious amounts of milk production, increase oxygen requirements substantially. As a result, the production of reactive oxygen species (**ROS**) such as O_2^-, OH^-, H_2O_2, and lipid peroxy radical (**LOO$^-$**) increases. Oxidative stress results when ROS are produced faster than they can be neutralized by antioxidants (Sies, 1991). Oxidative stress usually occurs during the periparturient period (Ronchi et al., 2000) and may contribute to periparturient disorders (Brezezinska-Slebodzinska et al., 1994) and be associated with metabolic diseases (Ronchi et al., 2000). The predominant antioxidant in biological cell membranes is α-tocopherol. Heat stress may aggravate oxidative stress (Bernanucci et al., 2002). Feeding additional vitamin E as an antioxidant in the summer during the periparturient period may be needed due to the greater oxidative stress caused by elevated temperature and humidity.

Experimental Design

Objective was to evaluate vitamin E supplementation above NRC recommendations during the close-up dry period and during lactation to Holstein cows (36 primiparous and 34 multiparous) managed either in free-stall barns using fans and sprinklers to evaporatively cool the cows or in shaded outdoor lots without fans and sprinklers during the prepartum period. After calving, all cows were housed together in

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a free-stall facility equipped with fans and sprinklers. All-rac-alpha-tocopherol (DSM, Parsippany, NJ) was mixed with ground corn and dried molasses and top-dressed daily [2](100 g of mix/cow) on the first feeding of the day of the total mixed ration. Amounts of vitamin E supplementation were either 1,000 IU prepartum and 500 IU postpartum as recommended by the NRC (2001) for feeding of dairy animals or 3,000 IU prepartum and 2,000 IU postpartum (greater than current published recommendations for vitamin E). In summary, the 4 treatments were 1) prepartum shade only and NRC amounts of vitamin E, 2) prepartum shade only and a 3 to 4 fold increase in vitamin E supplement, 3) prepartum evaporative cooling and NRC amounts of vitamin E, and 4) prepartum evaporative cooling and a 3 to 4 fold increase in vitamin E supplement. The treatments started at 4 weeks prepartum and continued through 15 weeks of lactation. Measurements included heat stress responses prepartum, intake of feed, body weight, yields of milk and milk fat and protein. Blood was collected on days -30, -14, 3, 7, 14, 21, 28, 35, and 42 relative to calving for analyses. Cows were bred using timed artificial insemination at 46 and 64 ± 3 days in milk. Conceptuses were collected by uterine flushing 15 days after each AI and their length measured.

Results and Discussion

Prepartum Responses

During the prepartum period, the environmental temperature-humidity index (**THI**) averaged 74.8 ± 4.9 and cows were exposed to a THI greater than 70 during 85% of the day. Therefore the study was conducted during conditions of heat stress. Providing fans and sprinklers to one group of cows kept them cooler than the group only given shade. Prepartum evaporative cooling in free-stalls reduced vaginal temperature from 102.9 to 102.2^{o}F (mean of measurements taken regularly between noon and 7 p.m.), reduced respiration rates from 69 to 43 breaths per minute (measured at 3 p.m.), reduced plasma concentrations of nonesterified fatty acids (**NEFA**) from 0.28 to 0.14 mM, and increased intake of feed dry matter (**DM**) by 15%, from 8.3 to 9.5 kg/day across parities ($P < 0.05$). As expected, cows offered only shade were hotter, breathed faster, and ate less feed compared with cows provided shade, fans, and sprinklers. Vaginal temperatures, plasma NEFA concentrations, and DM intake were not affected by the amount of vitamin E top-dressed. Multiparous cows experienced greater oxidative stress compared with primiparous cows based upon greater plasma concentration of lipid hydroperoxides (3.08 vs. 2.48 uM). The larger body size and greater feed intake (9.7 vs. 8.1 kg/day) of the older cows likely caused them to experience greater oxidative stress.

Plasma concentrations of vitamin E were lowest at 3 days in milk (Figure 1). All treatment means at this time were at or below the minimum acceptable concentration for the periparturient dairy cow (3.0 to 3.5 ug/mL) as suggested by Weiss (1998) who based these minimum blood values upon animals having good neutrophil function and reduced clinical mastitis. Greater prepartum heat stress did not affect vitamin E

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concentrations at this early time postpartum but greater vitamin E supplementation increased plasma concentrations from 2.8 to 3.4 ug/mL ($P < 0.001$) at 3 days in milk. This difference was even greater at 7 days in milk being 3.1 vs. 4.0 ug/mL for normal compared with high vitamin E treatments, respectively. By 14 days in milk, all treatment group means surpassed 4.0 ug/mL with the exception of the multiparous cows in the cooled normal vitamin E group which only averaged 3.2 ug/mL. If using minimum plasma vitamin E concentrations stated by Bill Weiss at Ohio State University as a guideline, multiparous cows may benefit from supplementation of vitamin E above NRC recommendations during the first 2 weeks postpartum.

In this study, incidence of retained fetal membranes (RFM; membranes retained for more than 24 hours after parturition) was 8.6%, incidence of metritis (fetid, watery, uterine discharge during the first 12 days postpartum) was 18.6%, and incidence of clinical mastitis (first 6 weeks postpartum) was 14.3%. The incidence of these maladies were not affected by the amount of vitamin E top-dressed in the study. In other studies, giving more vitamin E had benefited reproductive health. Injecting 3,000 IU of vitamin E within 1 to 2 weeks of calving reduced the incidence of RFM of heifers (LeBlanc et al., 2002) and of all parities (Erskine et al., 1997). Although the incidence of RFM, metritis, or mastitis was not affected by the amount of vitamin E top-dressed in our study, the mean plasma concentrations of vitamin E were lower in cows afflicted with these maladies. Plasma concentration of vitamin E averaged across -14, 3, and 7 days relative to calving was 3.6 and 2.5 ug/mL ($P < 0.001$) for healthy and RFM cows, 3.7 vs. 3.2 ug/mL ($P = 0.03$) for healthy and metritic cows, and 3.7 vs. 3.2 ug/mL ($P = 0.03$) for healthy and mastitic cows, respectively. Dry matter intake during the last 14 days before calving did not differ between healthy and sick cows, being 9.9 and 9.8 kg/day for healthy and RFM cows for example.

Postpartum Responses

Milk Production and Composition and Feed Intake. The benefit of providing fans and sprinklers to multiparous cows during the close-up dry period was clear. Multiparous cows fed the recommended amount of vitamin E produced an average of 36.0 kg/day of 3.5% fat-corrected milk if provided only shade during the close-up dry period. However, if the multiparous cows were provided shade, fans, and sprinklers during the close-up dry period, they produced 39.9 kg/day of 3.5% fat-corrected milk, an increase of 3.9 kg/day due to prepartum cooling. This milk advantage due to cooling of multiparous cows during the close-up period was similar to the increase in milk production reported when multiparous cows were cooled for the whole dry period (Tao and Dahl, 2013). Feeding more vitamin E than currently recommended appeared to have the same positive effect on milk yield as prepartum cooling in our study. Multiparous cows offered shade only during the close-up period and fed the normal amounts of vitamin E produced an average of 36.0 kg/day of 3.5% fat-corrected milk. If the supplementation of vitamin E increased to 3,000 IU prepartum and 2,000 IU postpartum, average milk production increased to 39.5 kg/day, an increase of 3.5 kg/day. So milk production by multiparous cows was greatest under two very different management conditions. Milk yield by multiparous cows was the same using 1) evaporative cooling conditions plus supplementing recommended amounts of vitamin E and 2) shade only plus supplementing extra vitamin E. These increased amounts of milk yield were supported by increased amounts of feed DM intake; that is, multiparous cows ate more feed DM after calving if cooled in the close-up period or if fed more vitamin E and housed with only shade compared with shaded cows fed recommended amounts of vitamin E. Multiparous cows benefited from consuming more vitamin E only when provided shade alone during their late dry period in the summer season.

The story for prepartum cooling and vitamin E supplementation was much different for primiparous cows. Evaporative cooling during the close-up period of primiparous cows did not result in greater milk production or greater feed intake postpartum as it did for multiparous cows. The milk secreting cells of the mammary gland of primiparous cows may be more resistant to decreased cell proliferation due to heat stress compared with multiparous cows. The effect of supplementing more vitamin E to primiparous cows provided only shade was negative and the opposite of the positive effect on multiparous cows. For primiparous cows not cooled with fans and sprinklers prepartum, supplementing with more vitamin E reduced postpartum DM intake from 19.8 to 17.9 kg/day (a 1.9 kg/day decrease) and reduced yield of 3.5% fat-corrected milk from 27.7 to 22.5 kg/day, a decrease of 5.2 kg/day. As a result, the conversion of feed into 3.5% fat-corrected milk was decreased from 1.49 to 1.33 kg of milk per kg of feed. Feeding more vitamin E to evaporatively cooled primiparous cows in the dry period did not affect their postpartum intake of DM or their production of milk.

The opposite responses by primiparous and multiparous cows to additional vitamin E supplementation was unexpected. The reasons for the different responses may result from differences in stress between parities and therefore a difference in need for additional vitamin E. Multiparous cows appeared to be under greater stress than primiparous cows. After calving, the loss of body weight was greater (35 vs. 12 kg) and the time it took to recover the lost body weight was greater (15 vs. 5 weeks) for multiparous compared with primiparous cows (Figure 2). The average energy balance during the first 15 weeks postpartum also was less for multiparous compared with primiparous cows (-0.8 vs. 3.6 Mcal/day). This greater metabolic stress of multiparous cows is supported by the greater blood concentrations of NEFA (fat mobilized from adipose tissue; 0.36 vs. 0.21 mM) and the ketone body, beta-hydroxybutyric acid (BHBA; produced by the incomplete oxidation of mobilized fat; 0.65 vs. 0.53 mM). During the close-up dry period, the multiparous cows also seemed to be under greater stress. They had increased concentration of lipid hydroperoxides in their blood during the prepartum period. These lipid hydroperoxides form as a result of oxidative stress in the cells of the cow. Oxidative stress results from increased demand for oxygen under conditions described in the Background section of the paper. Excess free radicals form as a result of uncontrolled oxidative stress. These free radicals can react with lipids in cell walls. Vitamin E is stored in the lipid portion of cell walls, ready to react with free radicals so as to prevent damage and death to the cells. As vitamin E donates its hydrogens to free radicals to form harmless water and to stop lipid peroxidation, a vitamin E radical forms called tocopheroxyl radical. This vitamin E radical itself can be dangerous to the cell if the vitamin E is not returned to its normal state with the help of vitamin C. Supplementing additional vitamin E can be a good thing if the cow is under additional oxidative stress or it may be a bad thing if oxidative stress is not under control. In our study, the multiparous cows appeared to be under more stress compared to the primiparous cows and therefore benefited from consuming more of the important antioxidant vitamin E. The primiparous cows likely consumed more vitamin E than they required and may have experienced tissue damage at the cellular level resulting in lowered performance.

Mean concentration of milk fat was 3.60% across all treatments and all weeks of lactation. Neither prepartum cooling nor increased vitamin E supplementation affected milk fat concentration of primiparous cows (mean of 3.56%). For multiparous cows, the story was different. Cooling multiparous cows prepartum resulted in greater milk fat concentration, increasing from 3.52% to 3.73%. This agrees with others who reported improvements in milk fat % if cows were evaporatively cooled prepartum (Avendano-Reyes et al., 2006; do Amaral et al., 2009). Interestingly, the feeding of additional vitamin E to noncooled multiparous cows increased milk fat % to the same extent (from 3.52% to 3.73%) as if they had been evaporatively cooled. In other words, feeding additional vitamin E had the same positive effect on milk fat % as did prepartum cooling. Providing additional vitamin E during the late dry period when cows were only offered shade from the heat may have helped maintain good development of the mammary gland cells. Concentration of milk true protein (mean of 2.94%) was not affected by evaporatively cooling or by the amount of vitamin E supplemented.

Immune responses. The white blood cells are largely responsible for keeping the cow heathy. In this study, we examined the activity of one kind of white blood cells called neutrophils. Neutrophils travel to sites of infection through the blood stream in order to engulf and then kill the invading pathogens. We collected blood from the cows and added the bacteria, *E. coli*, to the blood to assess how effectively the neutrophils would destroy this pathogen. First we will look at the prepartum period. Providing extra vitamin E to shade-only primiparous cows reduced the proportion of neutrophils killing bacteria from 49 to 41%. However the opposite occurred for shade-only multiparous cows. Feeding extra vitamin E tended to increase the proportion of neutrophils killing bacteria from 33% to 43% (vitamin E by cooling by parity interaction, $P = 0.07$). This response of benefit to extra vitamin E by multiparous cows and of harm to primiparous cows is similar to what occurred with milk production. What about the postpartum period? When NRC amounts of vitamin E were fed, a greater proportion of the blood neutrophils tended to engulf and kill the bacteria in the postpartum period if the cows were evaporatively cooled prepartum than if the cows were not evaporatively cooled prepartum (39.5% vs. 33.7%). These positive results due to prepartum cooling agree with those of do Amaral et al. (2011). However, if vitamin E was fed above NRC recommendations, the proportion of neutrophils that killed bacteria tended to be reduced from 39.5% to 33.2% when cows were evaporatively cooled but the proportion was not affected under greater heat stress conditions (vitamin E by cooling interaction, $P = 0.09$). Feeding extra vitamin E may have acted as a prooxidant and reduced the killing activity of neutrophils when cows were under less stress.

Conceptus Length. 15 days after insemination, conceptuses were collected from 32 animals and measured for length. The current thinking is that conceptus length may be a good indicator of development and that longer conceptuses are more likely to survive and go on to become calves. Providing more vitamin E to cows that had been exposed to greater heat stress prepartum resulted in longer conceptus length (28 vs. 63 mm) whereas conceptus length was shorter if cows were evaporatively cooled prepartum and fed more vitamin E (38 vs. 8 mm; VitE by cooling interaction, $P < 0.01$). The impact of feeding more vitamin E on development of the conceptus was dependent upon the prepartum environmental conditions in this small data set. Feeding excess vitamins E and C to mice also had negative effects on mice, reducing the percentage of viable fetuses (Tarin et al, 2002). Feeding more vitamin E to multiparous cows tended to double the length of the conceptus at 15 days of life whereas it tended to reduce the length of the conceptus of primiparous cows.

Summary and Conclusions

Daily intake of supplemental vitamin E was increased from 1,000 to 3,000 IU during the close-up prepartum period and from 500 to 2,000 IU postpartum for primiparous and multiparous cows housed with shade only or with shade, fans, and sprinklers during the last 4 weeks of pregnancy. Milk production and milk fat concentration of multiparous cows housed without fans and sprinklers in the prepartum period was negatively affected. However, both milk production and milk fat concentration were restored if cows were provided with more vitamin E. No benefit of extra vitamin E was detected if multiparous cows were evaporatively cooled prepartum. Production of fat-corrected milk of primiparous cows was reduced if they were fed vitamin E above NRC recommendations regardless of prepartum cooling method. Based upon lower plasma concentrations of NEFA, less loss of body weight, and less negative energy balance, primiparous cows were under less metabolic stress postpartum than were multiparous cows. Feeding 3 to 4 times the recommended amount of the antioxidant vitamin E to these lower-stressed cows may have caused vitamin E to form many tocopherol radicals that damaged cell membranes and hurt performance rather than act as an antioxidant and help performance as it did with the multiparous cows. The combination of increased thermal stress prepartum and metabolic stress due to greater milk production postpartum may have created a scenario in which the requirement for an antioxidant, vitamin E, was increased for multiparous cows.

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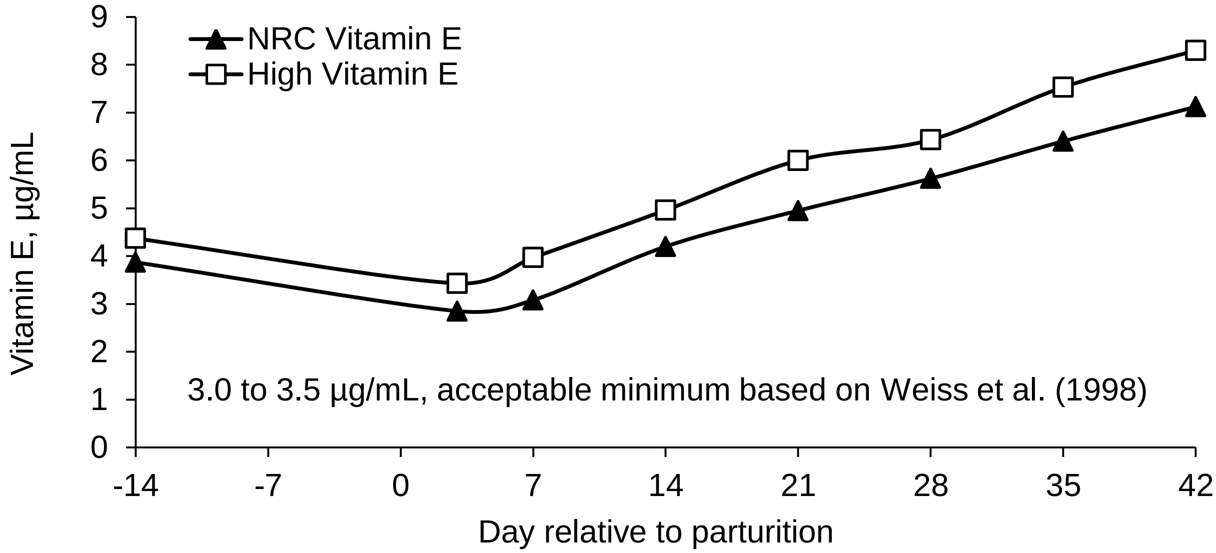

Figure 1. Concentrations of vitamin E in plasma of Holstein cows fed recommended amounts of vitamin E (1,000 IU/day close-up prepartum and 500 IU/day postpartum; "NRC Vitamin E") or 3,000 IU/day in the close-up and 2,000 IU/day in the postpartum periods ("High Vitamin E").

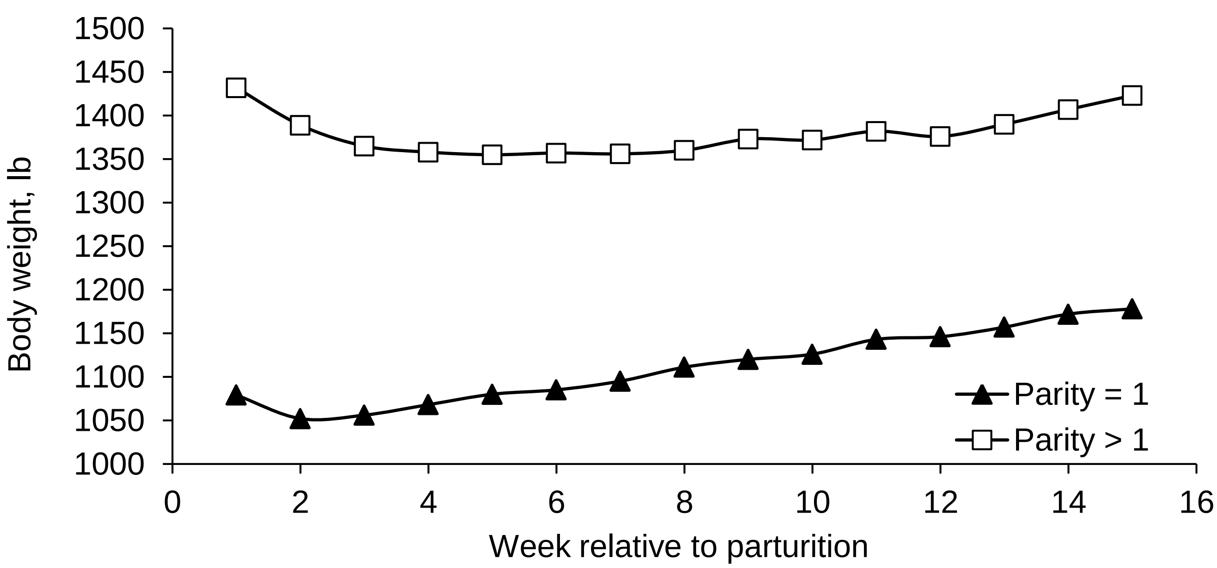

Figure 2. Body weight of primiparous and multiparous lactating Holstein cows from calving through 15 weeks of lactation.

SESSION NOTES